DCC is required for the development of nociceptive topognosis in mice and humans.

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Abstract: Avoidance of environmental dangers depends on nociceptive topognosis, or the ability to localize painful stimuli. This is proposed to rely on somatotopic maps arising from topographically organized point-to-point connections between the body surface and the CNS. To determine the role of topographic organization of spinal ascending projections in nociceptive topognosis, we generated a conditional knockout mouse lacking expression of the netrin1 receptor DCC in the spinal cord. These mice have an increased number of ipsilateral spinothalamic connections and exhibit aberrant activation of the somatosensory cortex in response to unilateral stimulation. Furthermore, spinal cord-specific Dcc knockout animals displayed mislocalized licking responses to formalin injection, indicating impaired topognosis. Similarly, humans with DCC mutations experience bilateral sensation evoked by unilateral somatosensory stimulation. Collectively, our results constitute functional evidence of the importance of topographic organization of spinofugal connections for nociceptive topognosis.

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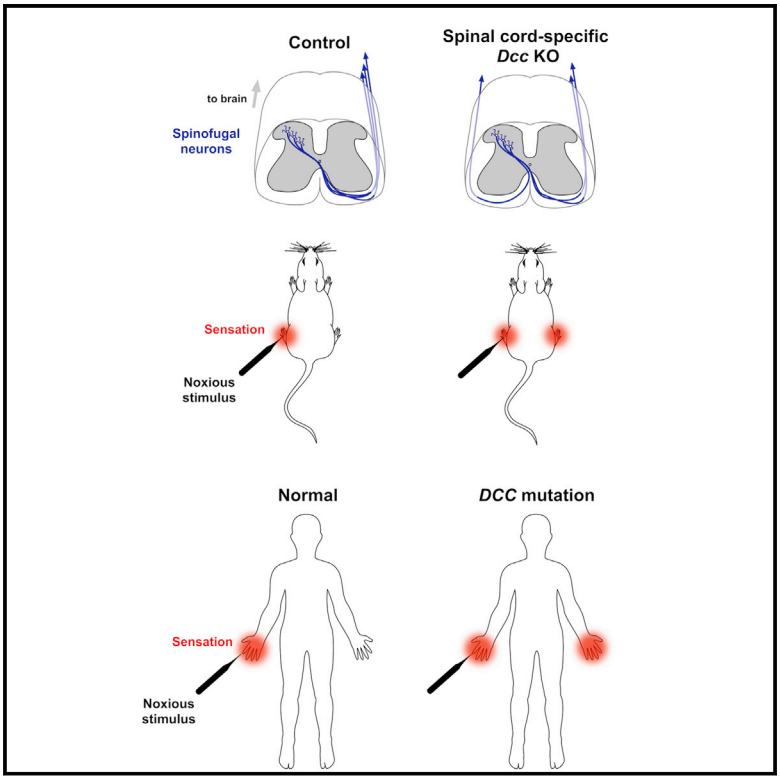
Graphical Abstract



Highlights

- Spinal cord-specific *Dcc* knockout mice have more ipsilateral spinothalamic connections
- Dcc^{SpKO} cortical activity evoked by noxious stimulation is changed
- Dcc^{SpKO} mice show somatotopically misdirected nocifensive behaviors
- *DCC* mutations in humans lead to mirroring of somatosensory stimuli

Authors

Ronan V. da Silva, Helge C. Johannssen, Matthias T. Wyss, ..., Bruno Weber, Hanns Ulrich Zeilhofer, Artur Kania

Correspondence

artur.kania@ircm.qc.ca

In Brief

Da Silva et al. show that the axon guidance receptor DCC is necessary for the lateralization of spinothalamic projections. Mice lacking *Dcc* in the spinal cord have abnormal somatosensory cortex activation in response to noxious stimulation and fail to accurately localize noxious stimuli. *DCC* mutations in humans lead to mirroring of somatosensory stimuli.

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Report

DCC Is Required for the Development of Nociceptive Topognosis in Mice and Humans

Ronan V. da Silva,[1,2] Helge C. Johannssen,[3,4] Matthias T. Wyss,[3,5] R. Brian Roome,[1,2] Farin B. Bourojeni,[1,2] Nicolas Stifani,[1] Ashley P.L. Marsh,[6,7] Monique M. Ryan,[7,8,9] Paul J. Lockhart,[6,7] Richard J. Leventer,[7,8,9] Linda J. Richards,[10] Bernard Rosenblatt,[11] Myriam Srour,[11] Bruno Weber,[3,5] Hanns Ulrich Zeilhofer,[3,4] and Artur Kania[1,2,12,13,*]

[1]Neural Circuit Development Laboratory, Institut de Recherches Cliniques de Montréal (IRCM), Montreal, QC, Canada
[2]Integrated Program in Neuroscience, McGill University, Montreal, QC, Canada
[3]Institute of Pharmacology and Toxicology, University of Zurich, Zurich, Switzerland
[4]Institute of Pharmaceutical Sciences, Swiss Federal Institute of Technology (ETH) Zurich, Switzerland
[5]Neuroscience Center Zurich, University and ETH Zurich, Zurich, Switzerland
[6]Bruce Lefroy Centre for Genetic Health Research, Murdoch Children's Research Institute, Royal Children's Hospital, Parkville, VIC, Australia
[7]Department of Paediatrics, University of Melbourne, Parkville, VIC, Australia
[8]Department of Neurology, University of Melbourne, Royal Children's Hospital, Parkville, VIC, Australia
[9]Neuroscience Research Group, Murdoch Children's Research Institute, Parkville, VIC, Australia
[10]The University of Queensland, Queensland Brain Institute and School of Biomedical Sciences, St. Lucia, Brisbane, QLD, Australia
[11]Division of Pediatric Neurology, Montreal Children's Hospital, McGill University Health Centre (MUHC), and Departments of Pediatrics, Neurology and Neurosurgery, McGill University, Montreal, QC, Canada
[12]Department of Anatomy and Cell Biology, Division of Experimental Medicine, McGill University, Montreal, QC, Canada
[13]Lead Contact
*Correspondence: artur.kania@ircm.qc.ca
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SUMMARY

Avoidance of environmental dangers depends on nociceptive topognosis, or the ability to localize painful stimuli. This is proposed to rely on somatotopic maps arising from topographically organized point-to-point connections between the body surface and the CNS. To determine the role of topographic organization of spinal ascending projections in nociceptive topognosis, we generated a conditional knockout mouse lacking expression of the netrin1 receptor DCC in the spinal cord. These mice have an increased number of ipsilateral spinothalamic connections and exhibit aberrant activation of the somatosensory cortex in response to unilateral stimulation. Furthermore, spinal cord-specific *Dcc* knockout animals displayed mislocalized licking responses to formalin injection, indicating impaired topognosis. Similarly, humans with *DCC* mutations experience bilateral sensation evoked by unilateral somatosensory stimulation. Collectively, our results constitute functional evidence of the importance of topographic organization of spinofugal connections for nociceptive topognosis.

INTRODUCTION

Acute pain functions as a warning about existing or potential tissue damage. Nocifensive behaviors rely on accurate stimulus localization, or topognosis, which has been proposed to be mediated by somatotopically organized neural connections that allow the nervous system to link stimulus quality to its location (Kenshalo and Isensee, 1983). Ascending pathways that connect the spinal cord to the brain have been proposed to be a major component of such circuits, but because their molecular and genetic handles remain elusive, the extent of their contribution to nociceptive topognosis remains unknown.

Somatotopic maps are characterized by topographically organized neuronal connections linking sensory neurons innervating adjacent locations on the surface of the body, with neural circuits located in adjacent regions of the thalamus (Mountcastle and Henneman, 1952) and the cortex (Penfield and Boldrey, 1937). One fundamental organizing principle of such maps is that sensory information from one side of the body is processed by rostral neural circuits contralateral to it, as a result of second-order ascending connections crossing the nervous system midline. In the case of nociception, for instance, spinothalamic commissural neurons postulated to relay topognostic information innervate the contralateral somatotopically organized ventral posterolateral nucleus (VPL) of the thalamus (Davidson et al., 2010; Guilbaud et al., 1980). Whether such commissural organization contributes to normal nociception remains unresolved.

Commissural axon guidance at the level of the spinal cord is an extensively well-studied subject (Chédotal, 2014). Among others, netrin1 signaling via its receptor DCC has been implicated in promoting the growth of axons across the midline (Fazeli et al., 1997; Keino-Masu et al., 1996). We have thus hypothesized that manipulating *Dcc* expression would allow us to change somatosensory circuit topographic organization at the level of commissural connectivity, allowing us to make inferences about the functional relevance of such organization. To

this end, we created mice lacking *Dcc* expression exclusively in the spinal cord (Dcc^{SpKO}), which exhibited increased numbers of ipsilateral spinothalamic projections. Such mice also showed changes in both the extent and timing of somatosensory cortex activation following peripheral noxious stimulation. Furthermore, although Dcc^{SpKO} mice displayed grossly normal nocifensive behaviors, these were directed toward somatotopically inappropriate locations, indicating a deficit in nociceptive topognosis. Extending these data, we also found that humans with *DCC* mutations exhibit sensory mirroring. Together, our findings provide a genetic handle on the relationship between topographic organization of spinofugal connections and topognosis.

RESULTS

Increased Ipsilateral Spinothalamic Connectivity in Spinal Cord-Specific *Dcc* Mutant Mice

We crossed the brain-sparing *Hoxb8::Cre* driver to the null and Cre-excisable alleles of *Dcc* (Dcc^{-} and Dcc^{f}, respectively) to produce *Hoxb8::Cre;* $Dcc^{f/-}$ (Dcc^{SpKO}) as well as control $Dcc^{f/+}$, $Dcc^{f/-}$, and *Hoxb8::Cre;*$Dcc^{f/+}$ littermates (Fazeli et al., 1997; Krimpenfort et al., 2012; Witschi et al., 2010). By embryonic day (E) 11.5, we observed a complete loss of DCC protein in the lumbar spinal cord of Dcc^{SpKO} embryos, but its expression was spared in the upper cervical spinal cord, where *Hoxb8::Cre* is not expressed (Figure 1A). At E14.5, when commissural axons have normally completed their midline crossing, we observed a 42.6% reduction in the thickness of Dcc^{SpKO} ventral commissure bundle compared to controls ($p < 0.01$). There were no changes in spinal cord thickness in the dorsoventral axis, but ventral funiculus thickness was significantly reduced ($p = 0.68$ and $p = 0.013$, respectively; Figures 1B and 1C), suggesting impaired midline crossing. The extent of commissure reduction was comparable with that reported in *Dcc* null mice (Xu et al., 2014). Dcc^{SpKO} mice were viable and grossly normal but displayed a hopping gait, as reported for the *Dcc* hypomorphic mutation (unpublished data; Finger et al., 2002).

We next focused our attention on the mostly commissural spinothalamic neurons that are proposed to relay topognostic information (Davidson et al., 2010; Guilbaud et al., 1980). *Hoxb8::Cre* expression has been reported to initiate at E9.5, which precedes the birth of the earliest born spinothalamic neurons (Beal and Bice, 1994). Moreover, we confirmed in adult animals that *Hoxb8::Cre* is expressed in virtually all lumbar spinothalamic neurons during their development (Figure S1). To assess whether spinothalamic neurons rely on DCC to innervate the contralateral thalamus, we labeled them in adult Dcc^{SpKO} and control mice by unilateral injection of FluoroGold (FG) targeting the VPL, the main target of spinothalamic neurons (Figures 1D and 1E). In the upper cervical spinal cord, we observed the same proportion of ipsilateral versus contralateral spinothalamic neurons in controls and mutants (Figures 1F and 1G). However, at lumbar levels of Dcc^{SpKO} mice, 36.5% of labeled spinothalamic neurons were located ipsilateral to the injection site, compared with only 3% in controls ($p < 0.01$; Figures 1H and 1I).

Nociceptive stimuli may also activate low-threshold receptors, which could be potentially involved in topognosis. Low-threshold mechanoceptive signals are relayed to the VPL contralaterally and in a somatotopic fashion via the dorsal column-medial lemniscal pathway (Ma et al., 1986). Unilateral retrograde tracer injection into the thalamus did not reveal any abnormal thalamic innervation of dorsal column nuclei (DCN) neurons in Dcc^{SpKO}, in agreement with lack of *Hoxb8::Cre* expression in the brainstem (Figure S2).

Normal Spinal Nociception in Dcc^{SpKO} Mice

We next examined the spinal nociceptive circuitry and spinal-mediated nociceptive reflexes of Dcc^{SpKO} mice. We investigated the central connectivity of peripheral mechanonociceptors by labeling them with isolectin B4 and of thermonociceptors by immunostaining for calcitonin gene-related peptide (CGRP) (Molliver et al., 1995). Except for a mild disorganization in the medial dorsal horn similar to that reported previously in *Dcc* knockout (Figure 2A, arrow; Ding et al., 2005), the innervation of lamina I by $CGRP^{+}$ axons and that of lamina II by $IB4^{+}$ axons did not differ between control and Dcc^{SpKO} animals (Figure 2A). Furthermore, the total number of $NeuN^{+}$ neurons, $Lmx1b^{+}$ excitatory neurons, or $Pax2^{+}$ inhibitory neurons was not significantly different between Dcc^{SpKO} and control mice (Figure 2B), suggesting that loss of *Dcc* does not influence nociceptive circuit development at the spinal level.

We then assessed the functionality of nociceptive spinal circuits in Dcc^{SpKO} mice. To do this, we analyzed the upregulation of Fos expression in the lumbar dorsal horn of control and Dcc^{SpKO} animals in response to intraplantar hindpaw injection of 5% formalin (Hunskaar et al., 1985). The number of neurons with upregulated Fos expression on the injected side was not different between Dcc^{SpKO} and control animals, suggesting that the functionality of afferent sensory connections and local nociceptive spinal circuits were not affected by the loss of DCC (Figure 2C). Also, nociceptive reflex response thresholds to von Frey fiber mechanical stimulation (Chaplan et al., 1994) and radiant heat stimulation (Hargreaves et al., 1988) were unchanged in Dcc^{SpKO} mice compared with controls (Figure 2D), suggesting that loss of spinal *Dcc* expression does not change the sensitivity of mice to noxious stimuli applied to the hindlimb.

Abnormal Noxious Stimulus-Evoked Cortical Activity in Dcc^{SpKO} Mice

We next used intrinsic optical imaging (IOI) in the somatosensory cortex to map changes in cortical encoding of painful stimuli in Dcc^{SpKO} mice (Figures 3A and 3B; Table S1). In control animals, noxious electrical hindpaw stimulation evoked robust activation of the contralateral primary somatosensory cortex (S1; $p < 0.001$ compared with the ipsilateral hemisphere signal; Figures 3C and 3D). In contrast, Dcc^{SpKO} animals showed reduced contralateral S1 activation with no significant difference in integrated IOI signal between both hemispheres ($p = 0.08$; Figures 3C and 3D). This resulted in a reduced difference between contralateral and ipsilateral S1 in Dcc^{SpKO} animals compared with control mice ($p < 0.001$; Figure 3E).

Because IOI signals primarily reflect action potential firing (Polley et al., 1999b), it is possible that the contralateral signal is being reduced in Dcc^{SpKO} by inhibitory inputs originating from the hemisphere ipsilateral to the stimulus (Palmer et al., 2012). In order to elucidate the mechanism underlying this reduction,

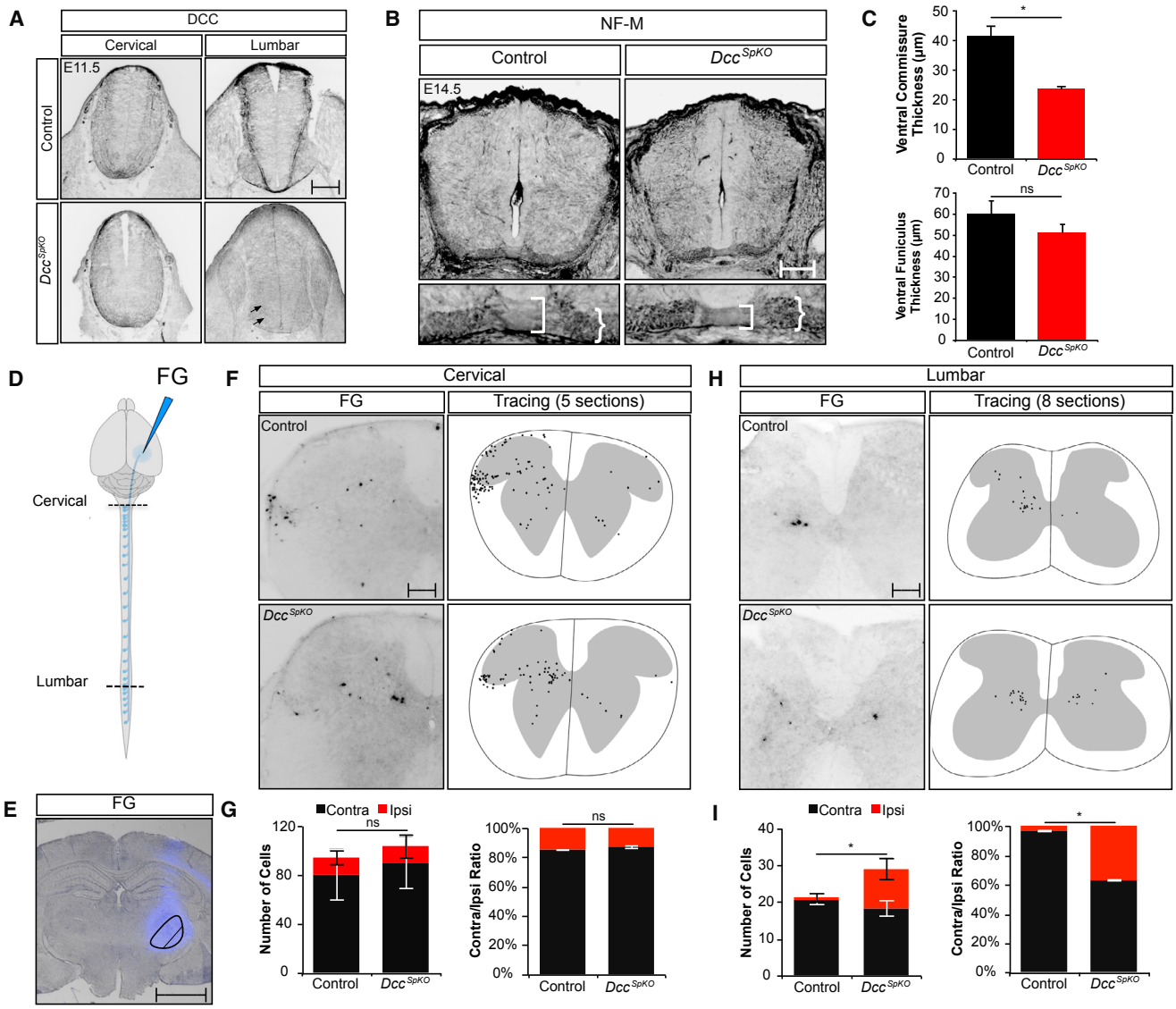

Figure 1. *Dcc*^{SpKO} Mice Have Reduced Ventral Spinal Commissures and Bilateral Spinothalamic Innervation

(A) DCC immunofluorescence in the cervical and lumbar spinal cord of E11.5 embryos. Inverted fluorescent signal. Arrows, commissural axons lacking DCC expression in *Dcc*^{SpKO} embryos. n = 3 embryos per group. Scale bar, 200 µm.

(B) Neurofilament M expression in mutant and control E14.5 lumbar spinal cord. Bottom, magnification of the ventral commissure (square brackets) and the ventral funiculus (curly brackets). Scale bar, 500 µm.

(C) Measurement of ventral spinal commissure and ventral funiculus thickness. Mean ± SD. *p < 0.05; ns, not significant; Student's t test; n = 4 embryos per group.

(D) Thalamic injection of retrograde tracer FluoroGold (FG) and labeled spinothalamic neurons.

(E) A thalamic FG injection site. Outline, ventral posterior complex. Scale bar, 3 mm.

(F) FG^{+} cells in the cervical spinal cord at C1–C2 (inverted fluorescent signal). Left micrographs are sample sections, right panels are overlays of five sections. Scale bar, 100 µm.

(G) Quantification of labeled ipsilateral and contralateral cell numbers and percentage of contralaterally versus ipsilaterally projecting neurons. Mean ± SD. ns, not significant; Student's t test; n = 3 mice per group.

(H) Images from the animals in (F), showing FG-labeled spinothalamic neurons in the L4 segment (inverted fluorescent signal). Right, overlays of eight sections. Scale bar, 100 µm.

(I) Similar quantifications as in (G) for lumbar sections. Mean ± SD. *p < 0.05; ns, not significant; Student's t test; n = 3 mice per group.

we assessed cortical activation using fast voltage-sensitive dye imaging (VSDi) to measure stimulus-evoked membrane potential dynamics (Figure 3F; Table S2). In both control and *Dcc*^{SpKO} mice, VSDi revealed that unilateral noxious hindpaw stimulation triggered a rise in membrane voltage in both contralateral and ipsilateral S1 (Figures 3G and 3H). However, whereas control mice featured early activity exclusively in contralateral S1 (auc_{30ms} contralateral versus ipsilateral, p = 0.002), *Dcc*^{SpKO}

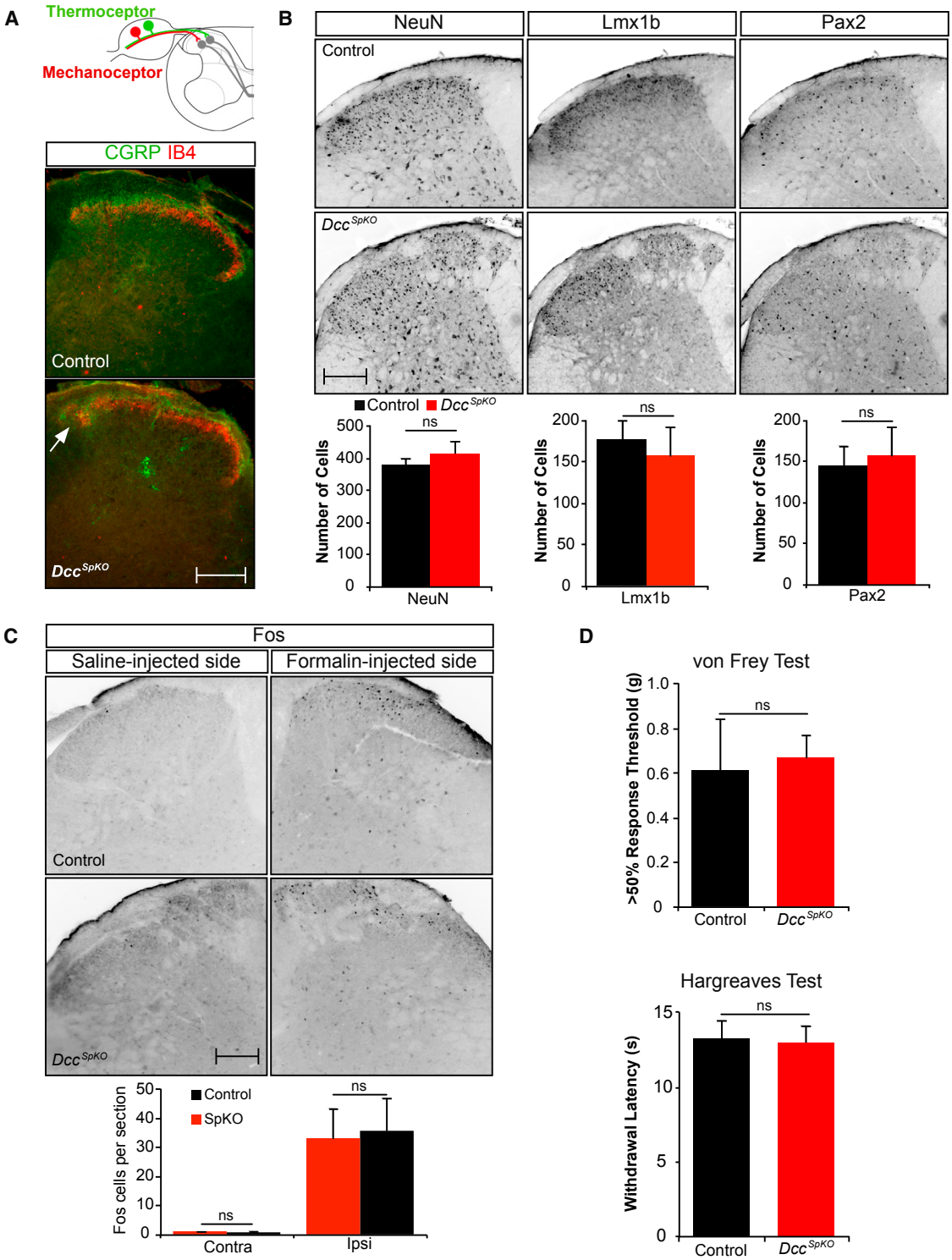

Figure 2. Spinal Nociceptive Circuit Anatomy and Function Is Unchanged in *Dcc*^{SpKO} Mice

(A) Representative images of adult spinal cord sections stained for markers of thermoceptor (CGRP, green) and mechanonociceptor (IB4, red) fibers. n = 3 mice per group.

(B) Adult lumbar dorsal horn sections showing all (NeuN), excitatory (Lmx1b^{+}), or inhibitory (Pax2^{+}) neurons. Inverted fluorescent signal. Mean labeled cells ± SD. *p < 0.05; ns, not significant; Student's t test; n = 4 control and 3 *Dcc*^{SpKO} mice.

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mice showed nearly simultaneous bilateral activation (auc_{30ms} contralateral versus ipsilateral, p = 0.2). In control mice, hindpaw stimulation evoked activation of contralateral S1 after a mean post-stimulus latency of approximately 20 ms (Figure 3H; see Experimental Procedures), followed by significantly delayed ipsilateral activation (p < 0.001; Figure 3I). In contrast, in Dcc^{SpKO} mice, the activation latency of each hemisphere upon unilateral stimulation was not significantly different (p = 0.2; Figure 3I), again indicating the presence of simultaneous inputs into both hemispheres. Consequently, the interhemispheric latency following unilateral noxious hindpaw stimulation was significantly shorter in Dcc^{SpKO} mice compared with control animals (p = 0.002; Figure 3J). Altogether, our results indicate that the increased number of ipsilateral spinothalamic projections in Dcc^{SpKO} mice leads to a premature activation of the ipsilateral S1, which, via callosal axons, could inhibit the S1 cortex contralateral to the stimulus, resulting in IOI signal decrease.

Dcc^{SpKO} Mice Have Reduced Topognostic Accuracy

To study how the anatomical and functional changes observed in Dcc^{SpKO} mice relate to nociceptive topognosis, we injected 5% formalin into the plantar surface of one hindpaw and saline into the opposite paw and recorded the location of licking behavior over 50 min (Figure 4A; Movies S1 and S2). This behavior has been proposed to depend on the transmission of nociceptive information from the spinal cord to the brain via spinofugal projections, and thus we expected its impairment in Dcc^{SpKO} mice. The total licking time over the recorded period did not differ between Dcc^{SpKO} and control mice, suggesting that Dcc^{SpKO} mice perceived the stimulus to be as aversive as control mice (p = 0.66; Figure 4B). However, whereas control animals almost exclusively licked the formalin-injected compared with the saline-injected paw (91% and 1.8% of total time, respectively), Dcc^{SpKO} mice spent significantly less time licking the formalin-injected paw and more time licking the saline-injected paw (61% and 13% of total time, respectively), as well as the trunk (15%) and genitals (8.7%; p values in Figures 4B and 4C). This behavioral change is unlikely attributed to defects in motor circuits, because *Dcc* deletion is restricted to the spinal cord and did not impair the movement of the head and forelimbs (see Movies S1 and S2). We can therefore conclude that the observed changes in licking location are a result of an impaired perception of the stimulus, rather than a motor phenotype. Altogether, this experiment suggests that Dcc^{SpKO} mice perceive noxious stimuli with the same intensity as control mice but have an impaired ability to localize them.

DCC Mutation Impairs Topognosis in Humans

To complement the Dcc^{SpKO} mouse analyses, we examined whether *DCC* mutations also impair topognosis in humans. Human *DCC* mutations result in "mirror movements" (MMs), characterized by voluntary movements of one side of the body accompanied by involuntary movements of the opposite side (Srour et al., 2010; Welniarz et al., 2017), as well as isolated agenesis of the corpus callosum (ACC; Marsh et al., 2017). We assessed the perception of mechanical stimuli using von Frey filaments in individuals with heterozygous *DCC* mutations and recorded their reported sensation (Figure 4D). We assessed a total of ten individuals with MMs, seven of whom belonged to two different Australian families (Marsh et al., 2017) and three to a French-Canadian family (Srour et al., 2010). These individuals had varying levels of MMs with or without ACC, ranging from mild to strong phenotype (Table S3). When stimulated, four of ten subjects reported a sensation in the analogous contralateral, non-stimulated site (Figure 4D). This sensation ranged from barely perceptible to identical to stimulated side, depending on the subject and the body part stimulated. Curiously, some patients experienced such mirrored sensations only for particular body parts, such as the left thumb (patient 2-II-1) or right ankle (patient 4:7). Also of interest, the individuals presenting sensory mirroring had the most severe MM functional effects among all the tested individuals (Table S3), suggesting that mirrored sensation and MMs may depend on *DCC* function in a similar manner.

DISCUSSION

Pain is a complex experience, composed of sensory-discriminative and motivational-affective components (Casey and Melzack, 1968) thought to be relayed by two different ascending pathways: the lateral spinothalamic (or neospinothalamic) and the medial spinothalamic (paramedial) pathways, respectively (Lima, 2008). Dcc^{SpKO} mice display quantitatively normal nocifensive behaviors but directed toward somatotopically inappropriate locations, implying a dissociation of the sensory-discriminative and affective-motivational components of pain. Because *Dcc* mutation affects the laterality of spinofugal connections, we reasoned that the function of pathways with a low degree of lateralization is unlikely to be changed by *Dcc* mutation. For example, spinoparabrachial neurons, arguably the best studied spinofugal nociceptive population, do not follow an obvious commissural organization (Cameron et al., 2015; Feil and Herbert, 1995; Kitamura et al., 1993), whereas their parabrachial nucleus targets show only a crude topographic organization and have large receptive fields (Bourgeais et al., 2001). In fact, mounting evidence implicates this nucleus in processing the motivational-affective aspect of pain as well as coordination of autonomic responses (Campos et al., 2017; Gauriau and Bernard, 2002; Han et al., 2015). In contrast, loss of laterality of the neospinothalamic pathway is likely to affect its ability to encode stimulus location. Although without a specific manipulation of the spinothalamic neurons we cannot ascribe the observed behavioral defects exclusively to a particular pathway, we predict that a change in proportion of commissural versus ipsilateral projections would primarily affect the function of

(C) Fos expression in L4 dorsal horn sections of animals stimulated with 5% intraplantar hindpaw formalin or saline injection. Mean ± SD. ns, not significant; Student's t test; n = 3 mice per group.

(D) Response thresholds to mechanical (von Frey) or thermal (Hargreaves) noxious stimulation. Mean ± SD. ns, not significant; Student's t test; n = 9 mice per group (von Frey test) and n = 6 control and 4 Dcc^{SpKO} mice (Hargreaves test). Scale bars, 200 μm.

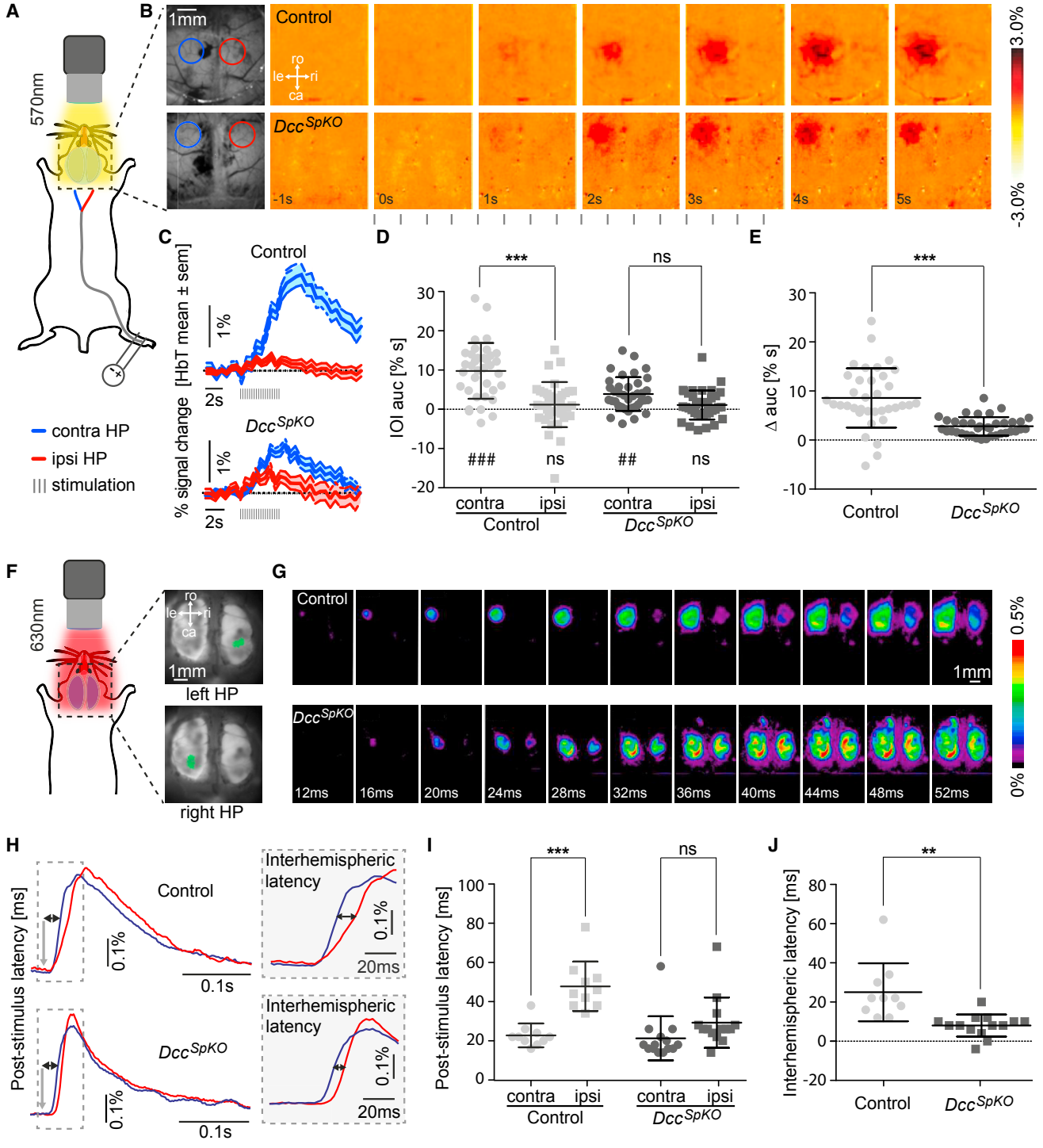

Figure 3. Cortical Noxious Stimulus-Evoked Activity Changes in *Dcc*^{SpKO} Mice

(A) Experimental setup used for bilateral imaging of stimulus-evoked cortical intrinsic optical signals.

(B) Circles at left show approximate regions of interest (ROIs) used for signal extraction in the contralateral (blue) and ipsilateral (red) hindpaw area of S1. Time sequences show the observed relative changes in intrinsic optical signals evoked by right hindpaw stimulation. Vertical gray bars represent electrical stimulation. Time stamps are relative to stimulus onset.

(C) Average stimulus-evoked changes in total hemoglobin (HbT) signal over time. Contralateral signals are shown in blue, ipsilateral signals in red. Horizontal dashed lines indicate baseline. Solid lines and shaded areas represent mean and SEM, respectively, and stimulation is illustrated by vertical gray bars.

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neospinothalamic connections, while sparing the paramedial system.

Our retrograde labeling experiments suggest that in Dcc^{SpKO} mice, spinothalamic axons from each body side converge on the same VPL nucleus, raising the question of whether somatotopic maps representing each body side can coexist in one thalamic hemisphere and how this might affect their function. A recent study showed that trigeminothalamic (TT) axons lacking *Robo3* innervate the thalamus in a bilateral manner (Renier et al., 2017): TT afferents originating from each side of the body segregated into distinct domains of both ventral posteromedial (VPM) nuclei, similar to ocular dominance columns (Hubel and Wiesel, 1969) or retinotectal input segregation in three-eyed frogs (Constantine-Paton and Law, 1978). It would be reasonable to assume that this might also be the case for lumbar spinothalamic neuron innervation of the VP thalamus in Dcc^{SpKO} animals. In view of this, one possible explanation for the erosion of nociceptive topognosis precision in Dcc^{SpKO} mutants is that the body maps in each thalamic or cortical hemispheres become fragmented, with a limited number of neurons processing pain localization, whose numbers now fall below a critical threshold required for accurate localization.

Direct functional insights into the connectivity and function of nociceptive circuits in Dcc^{SpKO} mice are provided by our two complementary *in vivo* S1 cortex imaging experiments. S1 is a part of the pain matrix, encoding for sensory stimulus location and intensity and is acutely activated by noxious stimulation (Bushnell et al., 1999; Gross et al., 2007; Cichon et al., 2017). Using IOI in anesthetized mice, we observed predominantly contralateral S1 activation elicited by noxious stimulation in control mice, in line with reports of optical hemodynamic imaging the cortical representation of sensory stimuli in rodents (Devor et al., 2008; Ma et al., 2016) and humans (Sato et al., 2002). Because hemodynamic signals are thought to arise following presynaptic action potential firing and postsynaptic integration (Logothetis et al., 2001; Polley et al., 1999a), the strong reduction in interhemispheric signal difference in Dcc^{SpKO} mice may reflect similar levels of spinothalamic input to both hemispheres.

Results from VSD imaging experiments reveal noxious stimulus-evoked initial contralateral S1 activation, followed by delayed ipsilateral S1 activation. This sequence of bilateral S1 activation resembles cortical activation evoked by innocuous touch observed with VSD imaging in behaving mice (Ferezou et al., 2007) but differs from functional imaging and somatosensory-evoked magnetic field studies in awake, attentive humans, in whom essentially only a contralateral S1 activation has been described (Omori et al., 2013; Talbot et al., 1991). Unilateral noxious stimulation of Dcc^{SpKO} mutant mice also results in bilateral activation of S1, but in contrast to controls, the ipsilateral activation follows the contralateral activation more rapidly. Given the increased incidence of ipsilateral spinothalamic connections in Dcc^{SpKO} mutants, the bilateral S1 signal can be explained by bilateral and simultaneous activation of thalamocortical afferents. Furthermore, we propose that in Dcc^{SpKO} mice, interhemispheric communication is also changed at cortical levels. Bilateral sensory stimulation potently inhibits pyramidal cell activity initially evoked only in contralateral S1 via commissural GABAergic signaling (Palmer et al., 2012). The decreased interhemispheric latency in Dcc^{SpKO} mice could likewise lead to effective cross-inhibition within a shorter time window, in line with the overall lower IOI amplitudes in Dcc^{SpKO} mutants.

In contrast to Dcc^{SpKO} mice, *DCC* mutant humans perceive the stimulus in the somatotopically appropriate location but on both sides of the body. What is evident from our analysis is the variability of penetrance of mirrored sensation whereby two individuals carrying the same mutation show remarkably different phenotypic severity (patients 2-II-1 and 2-III-3, for example) (Marsh et al., 2017). Callosal agenesis also does not apparently correlate with somatosensory mirroring: some of the patients with complete ACC were still able to localize noxious stimuli normally, suggesting that this structure is irrelevant for nociceptive topognosis. On the other hand, there appears to be a correlation between the severity of MMs and mirrored sensations, suggesting a shared mechanism of development between motor and sensory circuits. Conducting functional imaging experiments in humans with mirrored sensation might also extend lesion studies that highlight the importance of specific brain structures in different aspects of nociception. For example, blindtouch, in which somatosensory stimuli are perceived but not localized, is caused by thalamic lesions arguing for the importance of this structure for human topognosis (Halligan et al., 1997; Rossetti et al., 1995).

EXPERIMENTAL PROCEDURES

Further details and an outline of resources used in this work can be found in Supplemental Experimental Procedures.

Mouse Colony Management and Maintenance

All experiments involved mice from both sexes. Animal usage protocols were reviewed and approved by the Animal Care Committee of Institut de Recherches Cliniques de Montréal (IRCM) and by the veterinary office of the

(D) Scatterplots showing the distribution of integrated HbT signal magnitudes. n = 4 animals per group. ***p < 0.001 between groups; ###p < 0.001 to baseline; ##p = 0.002; ns, not significant. Data are presented as mean ± SD.

(E) Differences in integrated HbT signal between contralateral and ipsilateral hemispheres. n = 4 animals per group. ***p < 0.001. Data are presented as mean ± SD.

(F) Schematic of bilateral imaging of stimulus-evoked changes in membrane voltage. Early VSD signals observed in S1 following hindpaw stimulation are false-colored in green.

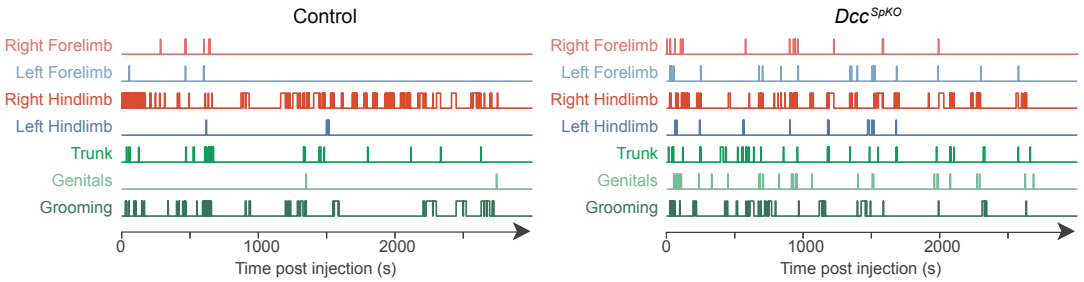
(G) Exemplary time courses depicting cortical activation triggered by right hindpaw stimulation. Time stamps are relative to stimulus onset.

(H) Example traces extracted from the S1 hindpaw area. Horizontal double arrows indicate latency between stimulation (vertical gray arrow) and half-maximal signal rise. Insets, higher magnification to illustrate the interhemispheric latency (horizontal double arrows) following unilateral hindpaw stimulation.

(I) Scatterplot of post-stimulus latencies. n = 4 animals per group. ***p < 0.001; ns, not significant. Data are presented as mean ± SD.

(J) Scatterplot showing differences in interhemispheric latencies between genotypes. n = 4 animals per group. **p < 0.01. Data are presented as mean ± SD.

A

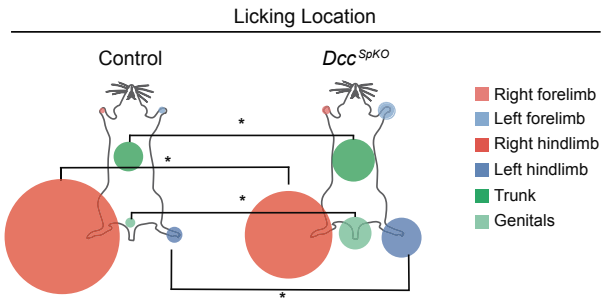


B

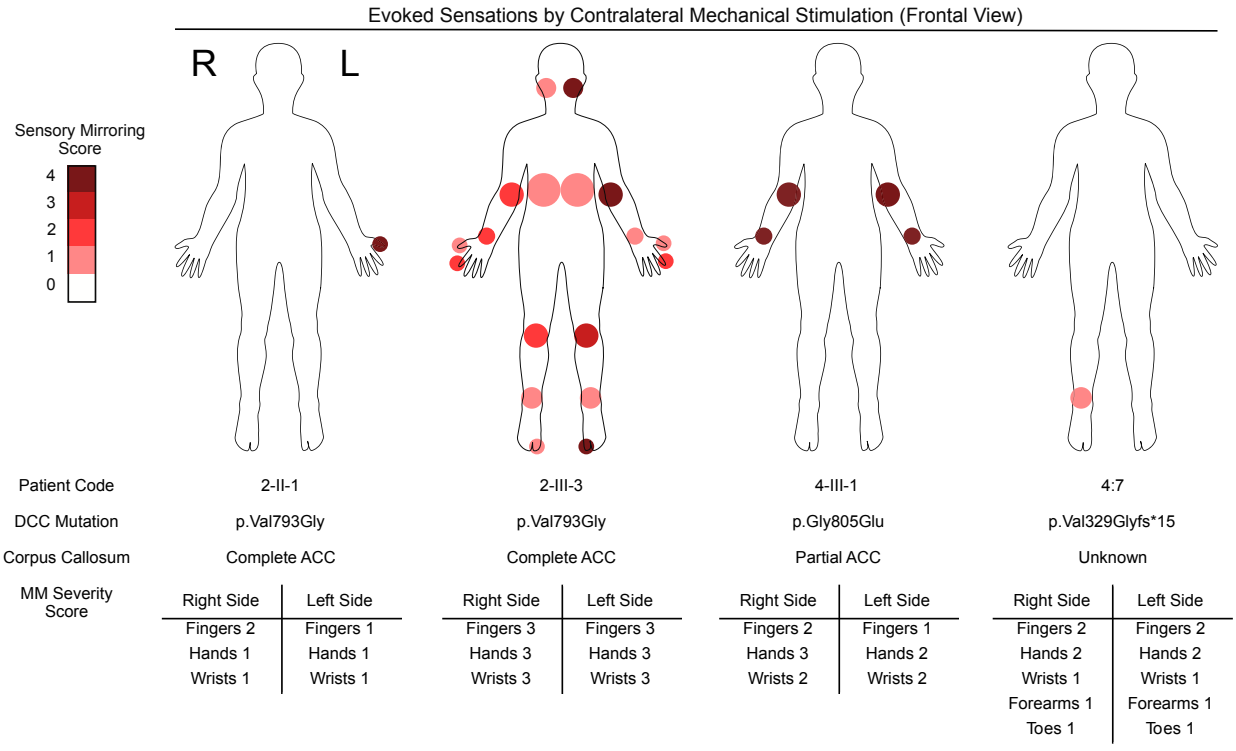
Licking Time (s)

	Control	*Dcc* ^{SpKO}	p
Right Forepaw	1.54 ± 1.45	3.76 ± 3.64	ns
Left Forepaw	2.46 ± 3.22	14.07 ± 2.55	ns
Right Hindpaw	693.68 ± 416.36	389.29 ± 318.84	*
Left Hindpaw	13.37 ± 10.63	83.52 ± 109.42	*
Trunk	45.76 ± 13.29	95.35 ± 40.41	*
Genitals	5.16 ± 2.52	56.02 ± 38.31	*
TOTAL	761.98 ± 412.77	642 ± 408.25	ns

C



D



	2-II-1	2-III-3	4-III-1	4:7
Patient Code				
DCC Mutation	p.Val793Gly	p.Val793Gly	p.Gly805Glu	p.Val329Glyfs*15
Corpus Callosum	Complete ACC	Complete ACC	Partial ACC	Unknown

MM Severity Score

Right Side	Left Side
Fingers 2	Fingers 1
Hands 1	Hands 1
Wrists 1	Wrists 1

Right Side	Left Side
Fingers 3	Fingers 3
Hands 3	Hands 3
Wrists 3	Wrists 3

Right Side	Left Side
Fingers 2	Fingers 1
Hands 3	Hands 2
Wrists 2	Wrists 2

Right Side	Left Side
Fingers 2	Fingers 2
Hands 2	Hands 2
Wrists 1	Wrists 1
Forearms 1	Forearms 1
Toes 1	Toes 1

Figure 4. Impaired Nociceptive Topognosis in *Dcc*^{SpKO} Mice and Somatosensory Defects in *DCC* Mutant Individuals with MMs

(A) Tracking of licking behavior in response to right hindpaw 5% formalin injection and left hindpaw saline injection in two representative animals. Each vertical line indicates a licking event.

(B and C) Average behavior times as mean ± SD (B) and a graphical representation of the average time spent licking each body part (circle area proportional to licking time) (C). *$p < 0.05$; ns, not significant; one-way ANOVA followed by Bonferroni post hoc paired comparison. n = 5 mice per group.

(D) Diagrams representing intensity of mirrored perception upon stimulation of contralateral side in human subjects bearing *DCC* mutations. Sensory mirroring scoring: 0, none; 1, barely discernible but present; 2, present but weaker than stimulated side; 3, strong but not complete; 4, completely mirrored. The MM severity score was based on the Woods-Teuber scale (Woods and Teuber, 1978).

Canton of Zurich, Switzerland. Mice were considered adult at an age of 2–6 months.

Human Subjects

The Royal Children's Hospital Human Research Ethics Committee approved the experiments conducted with Australian patients (project 28097). Four members of family 2 (II-1, a 50-year-old woman, III-1, an 18-year-old boy, III-2, a 16-year-old boy and III-3, a 15-year-old boy, all Caucasian) and three members of family 4 (II-1, a 45-year-old woman, III-1, a 12-year-old boy and III-2, a 14-year-old girl, all Caucasian) (Marsh et al., 2017) were assessed.

The McGill University Heath Center Research Ethics Committee approved the experiments involving Canadian patients. Informed consent was obtained from subjects. Three subjects were tested initially, all belonging to the same French-Canadian family (Srour et al., 2010): 3:3, a 66-year-old man, 3:14, a 54-year-old woman and 4:7, a 26-year-old man. All three individuals have congenital MMs (Woods-Teuber scale 3; Woods and Teuber, 1978) and bear the same *DCC* mutation.

Quantification and Statistical Analysis

Statistical analyses were performed using either Microsoft Excel or GraphPad Prism 6.0.

SUPPLEMENTAL INFORMATION

Supplemental Information includes Supplemental Experimental Procedures, two figures, three tables, and two movies and can be found with this article online at https://doi.org/10.1016/j.celrep.2018.01.004.

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AUTHOR CONTRIBUTIONS

Conceptualization and Methodology, R.V.S. and A.K.; Investigation – Mouse Model, R.V.S., H.C.J., M.T.W., F.B.B., and R.B.R.; Investigation – Human data acquisition and analysis, A.P.L.M., P.J.L., L.J.R., R.J.L., M.S., B.R., and M.M.R.; Writing – Original Draft, R.V.S. and A.K.; Writing – Review & Editing, R.V.S., H.C.J., M.T.W., N.S., H.U.Z., A.P.L.M., P.J.L., R.J.L., L.J.R., M.R., M.S., B.W., and A.K.; Software, N.S.; Supervision and Funding Acquisition, A.K., B.R., and H.U.Z.

DECLARATION OF INTERESTS

The authors declare no competing interests.

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